



Oncology Cytogenetics at

The Christie **NHS**
NHS Foundation Trust

USER GUIDE v5.1

Amendments highlighted



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Introduction

Oncology Cytogenetics at The Christie is a specialist regional service, which provides genetic testing to aid diagnosis of leukaemia and other tumours, mainly to hospitals in Greater Manchester and the North West of England. Malignant diseases are often classified by their genetic abnormalities and certain cytogenetic tests are essential for the optimal diagnosis and treatment stratification of cancer patients. Increasingly, cancer drugs are being developed that target specific genetic lesions, which therefore require a predictive molecular test.

Handling approximately 5,000 referrals per year, we are one of the largest specialist cancer genetics units in the UK. The department is the nominated service for leukaemia cytogenetics testing for two local Cancer Networks and is part of the GMC Haematological Malignancy Diagnostics service. Working closely with the specialist Histopathology and Breast Tumour Receptor sections of Pathology, we provide FISH testing for the diagnosis of various solid tumours and are continually developing assays to expand the diagnostic FISH service. The laboratory supports The Christie and other local multidisciplinary team meetings and provides specialised testing for clinical trials.

The Oncology Cytogenetics laboratory is accredited by Clinical Pathology UK Ltd (ref. no. 2034), whose standards are compliant with ISO 15189. The laboratory also complies with professional standards issued by the Association for Clinical Cytogenetics (ACC) and participates in all appropriate EQA schemes (UKNEQAS). The comprehensive cytogenetics service is provided by a highly-skilled team of dedicated scientists and technologists who offer a timely, efficient and cost-effective analytical and genetic advisory service to clinicians, to aid the diagnosis and monitoring of leukaemia and solid tumours. All relevant staff are state registered and clinical scientists are formally trained in Clinical Cytogenetics and Molecular Cytogenetics. We are a founder member of the UK Cancer Cytogenetics Group (UKCCG).



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Hours of Operation

Monday to Friday

8.30am to 5pm

Weekends

There is no routine service at weekends. Samples requiring special attention should be arranged in advance.

Bank Holidays

The department is not routinely staffed on Bank Holidays. For urgent attention, contact The Christie switchboard (0161 446 3000). A letter is sent in advance detailing arrangements at Christmas and Easter.



Samples

Sample Types

Bone Marrow is the tissue of choice to investigate patients suspected of having leukaemia or related haematological neoplasms. Bone marrow aspirate specimens are routinely received although a bone marrow trephine specimen is an option if the marrow is fibrotic or otherwise difficult to aspirate.

Peripheral Blood can be sent if disease cells are present in sufficient numbers to allow cell culture. This is satisfactory for FISH studies in CLL if there is peripheral blood lymphocytosis.

Other fresh tissues can be analysed and lymph nodes, spleen, ascitic fluid, CSF and solid tumours are occasionally received. We do not currently have facilities for long-term culture for a comprehensive solid tumour karyotyping service.

Paraffin Embedded Tissue for FISH on solid tissue, such as lymphoma, breast, sarcoma or brain tumour patients. Please send 3µm tissue sections (see pg. 34).

Specimen Containers

The laboratory will provide containers to regular referrers for bone marrow and blood collection. These bottles contain heparinised tissue culture medium with antibiotics, to facilitate the transport of the small amount of bone marrow and avoid desiccation.

An allocation of specimen bottles will be issued at the beginning of each week/month based on the number of samples usually received. More bottles can be sent upon request, at any time, by hospital transport or by post.

In emergency, a blood tube containing lithium heparin can be used.

Use only heparinised containers.
Please DO NOT use other anticoagulants such as EDTA, which is toxic to cells.

Fresh solid tissues should be placed in one of our Transport Bottles or other a sterile liquid such as culture medium, Hank's balanced salt solution or saline.

The department is pleased to advise on the use of alternative specimen containers.



Dispatch of Samples

All sample bottles should be fully labelled and placed in a plastic specimen bag with request card in separate pocket. Samples should be packed in sufficient absorbent packing material to soak up the entire contents, in the event of leakage, and placed in a cardboard sample box or other recommended receptacle.

Samples sent through the post, taxi or other courier service should comply with Packaging Instruction 650 and regulation UN3373.

Any packaging should bear the UN3373 diamond mark and labelled "Biological Substance, Category B" in letters at least 6mm high (see below).



BIOLOGICAL SUBSTANCE, CATEGORY B

For more information go to

www.hse.gov.uk/aboutus/meetings/committees/acdp/050208/acdp88p6.pdf

Fresh samples should be sent to the laboratory as soon as possible, preferably on the day of collection. Samples not being sent immediately should be refrigerated overnight at 4°C and sent at the earliest opportunity the following day.

First class post is usually acceptable. At times, it may be necessary to send specimens to the laboratory by taxi, to avoid delays, especially approaching weekends and bank holidays

It is advisable that all Friday samples arrive on the day of collection to ensure that the samples are set up in culture before the weekend. Myeloma samples need to arrive before 2pm to allow time for cell separation. **We cannot receive High Risk samples on a Friday (see page 9).**

**Please send samples at the earliest opportunity.
It is advisable to telephone about any samples that could arrive at the laboratory late
in the day or out of hours.
The Duty Scientist may advise sending the sample the following day.**

SAMPLES DETERIORATE RAPIDLY IN HOT WEATHER. IF SAMPLES MUST TRAVEL A LONG DISTANCE IN HOT TEMPERATURES, PLEASE CONSIDER SENDING THE SAMPLE IN A REFRIGERATED BOX.



Request Cards

Please fill in all the patient demographics on the request card.

The reason for referral is important to determine which culture types need to be set up, which tests to perform, numbers of cells to analyse and sample prioritisation. All relevant clinical and haematological information and likely diagnosis can be included. If the patient is a participant of a **research trial**, it is important to give details as certain trials can have specific requirements, such as levels of analysis by cytogenetics and/or FISH.

The department operates a Specimen Acceptance Policy [LP-PathGen-Christie SpecAccept]. The following details are essential requirements for request cards and specimens:

Request Card	Specimen bottle
1. Patients full name and date of birth. 2. Hospital number and/or NHS number. 3. NHS number for external referrals. 4. Reason for referral/clinical information. 5. Specimen type. 6. Consultant name or initials and hospital. 7. Requestor's name and signature. 8. Date specimen was taken. 9. High risk status (if appropriate). 10. Private patient (if appropriate).	1. Patients full name, with hospital number (or NHS number) and/or date of birth. 2. Specimen type and site of specimen to distinguish multiple specimens. 3. High-risk label (if appropriate)

**Please use the current version of the request card.
Please see following image of version 3.0**



Request Form v3.0

Lab. No.				ONCOLOGY CYTOGENETICS REQUEST		The Christie NHS NHS Foundation Trust	
ONCOLOGY CYTOGENETICS, PATHOLOGY DEPARTMENT, THE CHRISTIE, MANCHESTER M20 4BX							
STICK PATIENTS IDENTITY LABEL HERE OR GIVE THE FOLLOWING DETAILS:							
HOSPITAL		CONSULTANT		SURNAME			
DATE TAKEN	TIME TAKEN	SPECIMEN		FIRST NAME			
CLINICAL DIAGNOSIS & RELEVANT DETAILS Current treatment: ☐ DIAGNOSIS/PRESENTATION ☐ REMISSION/FOLLOW UP ☐ ?RELAPSE/PROGRESSION ☐ STAGING ☐ TRIAL HIGH INFECTION RISK? YES / NO (please circle) DETAILS: If YES please attach sticker & give details				ADDRESS			
				POSTCODE			
				NHS No. _ _ _ _ / _ _ _ _ / _ _ _ _			
				DATE OF BIRTH _ / _ / _ _		HOSPITAL NUMBER	
DOCTORS SIGNATURE		PRINT DOCTORS NAME		OTHER REFERENCE No. (e.g. Path No)		SEX	NHS PRIVATE Please tick
☐ MANDATORY INFORMATION							

LF - CG - Christie - Request Form v3.0

In submitting this sample, the clinician confirms that consent has been obtained for testing and storage of the patient material

All blue shaded areas are considered mandatory and should be completed before sending the form



Policy for High Risk Samples

All samples from patients exposed to a dangerous infectious pathogen (ACDP category 3 or higher) will be considered a high infection risk. This includes known carriers, people with prior contact to infected individuals and other risk groups such as IV drug users.

All samples from patients at High Risk of infection referred for cytogenetic analysis should be identified to the laboratory. The sample and request card must be clearly labelled as High Risk.

HIV, Hepatitis B or Hepatitis C samples can be processed by prior arrangement, if cytogenetic analysis is critical to patient management.

All other samples at High Risk of infection (with ACDP category ≥ 3 pathogen) cannot be processed by the laboratory.

Samples at risk of infection with HIV, Hepatitis B or Hepatitis C, may be processed by the laboratory*. However, as the samples require special attention, we request that these are arranged in advance between the Oncology Cytogenetics laboratory and the referring clinician. In particular, High Risk samples cannot be processed over a weekend and samples can only be accepted on a Friday in exceptional circumstances. Special arrangements must be made for Friday samples. Full cytogenetic analysis will only be considered in circumstances where a result will directly influence patient management.

The culture of cells from HIV, Hepatitis B or Hepatitis C samples requires special attention in isolation conditions. Processing of these samples will therefore incur an additional charge to the referring department. Alternatively, uncultured specimens can be fixed for FISH only but a full cytogenetic result will not be possible.

Any samples at risk of infection with any other ACDP category 3 pathogen (or higher) will not routinely be processed by the laboratory.

Any sample of uncertain risk status of infection with ACDP category 3 pathogen, or if satisfactory arrangements cannot be made, will be disposed of by incineration.

HIV, Hepatitis B and Hepatitis C samples received where no arrangements can be made or without strong indication for cytogenetic analysis, will be fixed uncultured for possible FISH only. Consequently, a conventional cytogenetics result will not be possible.

In all instances, a record of the actions taken will be made and a report issued to the referring consultant.

*Revised Advice on Laboratory Containment Measures for work with Tissue Samples in Clinical Cytogenetics Laboratories [2001]



Policy of Consent for Testing and Retention of Samples

In submitting a sample to Oncology Cytogenetics, the clinician confirms that consent has been obtained for testing and storage of the patient material.

This material is tested and surplus retained for possible Oncology Cytogenetics use and only in connection with the original reason for referral. It will not be passed on to other parties or used for research or purposes other than the reason that they were originally referred.

The Oncology Cytogenetics department currently retains fixed cell suspensions from samples for 10 years. This allows for further testing of samples and is particularly useful for additional FISH tests or when testing a diagnostic sample is required to establish a FISH signal pattern to enable testing of subsequent post-treatment samples.

Used microscope slides from routine cytogenetic analysis are retained for 10 years.

A sample not analysed at the time of referral can be reactivated at any time, if required.

A full year of samples and slides are disposed of in the January following the completion of a full ten years of age.



Sample Prioritisation and Reporting Times

Sample prioritisation and reporting performance targets of the professional standards of the Association of Clinical Cytogenetics are used as minimum standards, including General Best Practice Guidelines, Haemato-Oncology Best Practice Guidelines, Guidelines for FISH Scoring in Oncology and disease-specific Guidelines for CML/MPN, AML/MDS and ALL.

Urgent referrals (Acute leukaemia and CML at diagnosis or possible relapse)

All acute leukaemia and chronic myeloid leukaemia cases at diagnosis will be treated as 'urgent' and 95% of cases will be reported within 14 calendar days. In practice, the majority of diagnostic cases are reported within the internal reporting time target of 7 days.

Rapid FISH tests (e.g. APL, Burkitt lymphoma)

95% will be reported in 3 working days. In practice, the majority of cases will have a verbal report available within 24 hours or in 2-3 working days, if paraffin-embedded.

Routine referrals for routine cytogenetic analysis

95% of all other referrals will be reported within 21 calendar days. The laboratory operates a 'priority' system whereby cases requiring quick attention or by special (telephone) request but which are not 'urgent' can be analysed out of turn.

In Abeyance

All samples that are not urgent and have an uncertain diagnosis will be held in abeyance, pending further information. Further details are requested on an interim report (by e-mail where available), which also permits you to suggest a priority level for the referral.

This is necessary because at the time of biopsy the diagnosis may not be known and chromosome analysis may not be required after bone marrow morphology is examined.

Consultants are requested to cooperate as fully as possible with this policy. This is to avoid unnecessary and labour intensive analytical work and helps the laboratory to process its large workload

PET FISH

Reporting time targets for paraffin-embedded tissue sections referred for FISH have been agreed with local service users as follows;

HER2 on breast tumours	7 days for FISH (total turnaround including IHC = 10 days)
Lymphoma	7 days
Sarcoma	7 days
Brain tumours	14 days

Certain diagnoses and special requests can be turned around more rapidly (see above). In the majority of cases PET FISH referrals are reported in a significantly shorter time.



Conventional Cytogenetic Analysis

About Cytogenetics

Cytogenetics is the microscopic study of chromosomes, which can show abnormalities that represent genetic defects in the DNA that they contain. These abnormalities can be numerical (loss or gains of chromosomes) or structural (e.g. translocations, inversions, deletions).

Chromosome abnormalities can confirm a clonal disease and can often suggest a more specific diagnosis or a prognosis. The abnormalities can be used to monitor remission and diagnose relapse, transformation or secondary disease. Increasingly, cytogenetic abnormalities indicate specific and targeted treatment regimes.

Cytogenetic Techniques

Conventional cytogenetic analysis relies on the culture of cells to produce metaphase chromosomes, where individual chromosomes can be visualised. Tissue therefore needs to be as fresh as possible with viable disease cells. Cells are processed and stained using 'banding' techniques to produce a karyotype.

Fluorescence in situ hybridisation (FISH) uses fluorescently labelled gene probes to detect specific gene sequences on the microscope slide. This can be used to confirm specific genetic abnormalities at the molecular level. FISH can be used with metaphase chromosomes but is also applicable to interphase cells and so cell culture is not always necessary. Different strategies are possible with multiple fluorochromes, which can be used in conjunction with other fluorescent labelling techniques.



Telephone Enquiries

Telephone enquiries are welcome. Cytogenetics' staff will be pleased to accept requests to process samples if required urgently to determine treatment or by specific appointments and will make every effort to make results available.

Reporting

Hard-copy Reports

Signed hard-copy reports will be sent to the referring consultant by first-class mail. Reports can be addressed to other designated persons, if requested to do so in writing by the consultant.

Policy for Faxing Reports

Oncology Cytogenetics is obliged to follow the Fax policy of The Christie NHS Foundation Trust. For security reasons and because it is time-consuming, the procedure can only be used occasionally and therefore only when urgent reports are required immediately.

All other reports will be sent by post/hospital transport and will usually be received within a day of despatch.

Faxing will entail;

- Ring the recipient before we send the Fax.
- Check with the recipient that the Fax number and the Fax are available.
- Ask the recipient to stand by the Fax machine.
- Fax the header sheet only first.
- The recipient must phone when the header sheet is received.
- When we receive confirmation by phone, the rest of the Fax with the report will be sent.

E-mailing Reports

The department has developed protocols for the e-mailing of encrypted reports to workplace (NHS) addresses of referring consultants and other designated staff in the care team. Reports, which are e-mailed directly from the laboratory database, will be a copy of the final signed paper report, which will also be despatched. Reports are encrypted by the Trust's secure e-mail portal, Ironport, and will require entry of a password for access. Please enquire if this service may be of use and you are able to supply an appropriate e-mail address.



Summary of Services Offered for Routine Cytogenetics and FISH

Disease group	Cytogenetics	FISH
Chronic Myeloid leukaemia (at diagnosis)	✓	✓ (BCR/ABL1)
Chronic myeloid leukaemia (follow-up)	✓ Screen for Ph plus additional abnormalities including for Ph -ve clones	✓
Acute myeloid leukaemia (at diagnosis)	✓	To confirm specific abnormality found by cytogenetics or as indicated by morphology, such as CBFB or RUNX1/RUNX1T1. del(5q), del(7q) and del(17p) [TP53] on failed specimens.
APL (at diagnosis)	✓	Rapid FISH for PML/RARA
MDS	✓	del(5q) and del(7q) on failed specimens.
ET	✓ on request	✓ (BCR/ABL1)
PRV	✓	✗
Myelofibrosis	✓	✗
CMML	✓	✗
Hypereosinophilia	✓	FIP1L1-PDGFRA. PDGFRB, FGFR1 if indicated.
ITP	✗	✗
Aplastic anaemia	✓	✗
BMT patients (sex-mismatched)	Only if % recipient cells is significant by FISH.	✓ (X/Y)
BMT patients (sex-matched)	✓ (if abnormal at diagnosis)	✓ (if abnormal at diagnosis)
All follow-ups (except CML)	✓ (if abnormal at diagnosis)	✓ (if abnormal at diagnosis)
Acute lymphoblastic leukaemia	✓	ALL panel: BCR/ABL1, MLL, ETV6/RUNX1 (paediatric). TCF3, Hypodiploid and hyperdiploid panels if indicated.
CLL/SLL	✗	✓ TP53 and ATM only
Lymphoma (on staging bone marrow aspirate)	✗ (on request, if lymphocytosis)	✗ (on request if lymphocytosis or if FISH abnormal on other tissue)
Lymphoma (on lymph node biopsy)	✓	✓ (if indicated)
Lymphoma (on paraffin embedded tissue)	N/A	✓ (if indicated)
Multiple Myeloma (confirmed) [not MGUS]	✓ on request	IGH@/FGFR3, IGH@/MAF, TP53 on CD138 selected cells Other genes on request.
Fanconi Anaemia	✓	MECOM (EVI1) FISH (BM) MECOM + 7q FISH (PB)



Summary of Services Offered for Routine Cytogenetics and FISH (cont.)

Disease group	Cytogenetics	FISH
Breast carcinoma	N/A	HER2 amplification status (on paraffin embedded tissue)
Oesophagogastric carcinoma	N/A	HER2 amplification status (on paraffin embedded tissue)
Oligodendroglioma and other brain gliomas	N/A	1p- & 19q- deletion status (on paraffin embedded tissue)
Sarcoma	N/A	Rearrangements of EWSR1, SS18, DDIT3, FUS, FOXO1, COL1A1-PDGFB, as indicated MDM2 amplification. (on paraffin embedded tissue)
Non-Small Cell Lung Cancer	N/A	ALK (on paraffin embedded tissue)
Mesothelioma	N/A	Homozygous deletion of CDKN2A (p16)
Melanoma	N/A	In development; please enquire



SERVICE SPECIFICATIONS AND INDICATIONS

- 1. CONVENTIONAL CYTOGENETIC TESTING AND FISH FOR HAEMATOLOGICAL MALIGNANCIES**
- 2. FISH ON PARAFFIN-EMBEDDED TISSUE FOR SOLID TUMOURS**



CHRONIC MYELOID LEUKAEMIA (CML) CYTOGENETICS

Introduction

The characteristic genetic abnormality associated with CML is the translocation t(9;22), which gives rise to the Philadelphia (Ph) chromosome. This distinguishes typical CML from Ph negative MPN and reactive marrows. Additional clonal chromosome abnormalities may be present that could change prognosis. Confirmation of Ph positivity is an absolute requirement for treatment with tyrosine kinase inhibitors (TKIs) such as imatinib. A small number of cases have normal cytogenetics with a cryptic BCR-ABL1 gene rearrangement or variant translocations involving other chromosomes.

Early cytogenetic response is the most important prognostic factor in CML. Consensus strategies for monitoring CML recommend that cytogenetic studies should be part of the periodic review, such as performing bone marrow cytogenetics every 3 months until complete cytogenetic remission is achieved. Reviewing cytogenetics every 12 months to establish Ph status and to detect Ph negative clones is also an option.

Additional clonal chromosome abnormalities in the Ph negative cell line are a recognised phenomenon in a significant proportion of CML patients treated with TKI. The majority of these patients are asymptomatic but a small number develop MDS, with monosomy 7 and trisomy 8 appearing to confer a higher risk^{2,3}.

~80% of cases at transformation to acute leukaemia show clonal evolution with recognised additional chromosomal changes.

Full cytogenetic analysis should always be performed if there is a change in clinical or morphological status, there is a rise in BCR-ABL1 transcripts or MDS is suspected^{1,4,5}.

Referrals

- A full karyotype at diagnosis to detect t(9;22) and any additional cytogenetic abnormalities. Bone marrow cytogenetics is essential at diagnosis, on a pre-treatment specimen, to demonstrate the translocation t(9;22). Diagnostic samples will be treated urgently and a result will be available within 7 days.
- FISH at diagnosis to detect BCR-ABL1 gene rearrangement. FISH is performed on all cases at diagnosis, for rapid confirmation of BCR/ABL1 status and to establish a signal pattern for future monitoring. FISH is also used to detect the BCR-ABL1 gene rearrangement in cases with normal cytogenetics and cryptic BCR-ABL1 in cases with variant translocations involving other chromosomes and in the small number of cases that fail to grow in culture.
- Post-treatment bone marrows screened for Ph by cytogenetics and FISH. Chromosome analysis is used to monitor the initial response to treatment. However, more sensitive methods would be required (such as RT-PCR) to monitor patients after cytogenetic remission is achieved. Post-treatment patients will be monitored for Ph positivity by conventional cytogenetics and the common abnormalities seen at transformation (see below). Ph negative cells are screened for common abnormalities found in Ph negative clones.
- Conventional cytogenetic analysis on peripheral blood, to monitor remission, is unlikely to be successful. However, peripheral blood neutrophils screened in post-treatment samples by FISH is a suitable surrogate marker of bone marrow cytogenetic status.
- Full karyotype at possible relapse or transformation for t(9;22) and additional abnormalities
- Bone marrow samples should be sent for cytogenetic studies if disease acceleration is suspected or if there is a rising level of BCR-ABL1 transcripts by RT-PCR.

Technical

Cytogenetic analysis is performed on cultured cells from fresh bone marrow. 20 cells will be fully analysed, according to standard procedures and best practice guidelines. Cells may contain cryptic abnormalities and minor clones not represented in the cultured cells, which microscopic analysis may not detect.



Sample requirements

Bone marrow aspirate specimens should be collected fresh into the supplied transport bottles or alternatively into other lithium heparin-containing tubes. Samples should be sent to the laboratory as soon as possible, preferably on the day of collection. Peripheral blood specimens from typical CML cases are useful for FISH testing but may not yield sufficient cells for full cytogenetic analysis.

Summary of services and reporting times

Test	Target Reporting Time (Calendar days)
Rapid FISH at diagnosis	3 working days
Cytogenetic analysis (karyotype)	7
Cytogenetic analysis and/or FISH post-treatment	21
Cytogenetic analysis and/or FISH at relapse or transformation	7

Further Information

The Oncology Cytogenetics laboratory is accredited by Clinical Pathology UK Ltd (ref. no. 2034). CPA standards are compliant with ISO 15189. The laboratory also complies with professional standards issued by the Association for Clinical Cytogenetics (ACC) and participates in all appropriate EQA schemes (UKNEQAS). Please see relevant sections of the Oncology Cytogenetics User Guide for full details.

References

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3. Jabbour E et al. Chromosomal abnormalities in Philadelphia chromosome negative metaphases appearing during imatinib mesylate therapy in patients with newly diagnosed chronic myeloid leukemia in chronic phase. Blood. 2007 Oct 15;110(8):2991-5.
4. Hughes T et al. Monitoring CML patients responding to treatment with tyrosine kinase inhibitors: review and recommendations for harmonizing current methodology for detecting BCR-ABL transcripts and kinase domain mutations and for expressing results. Blood. 2006 Jul 1;108(1):28-37.
5. British Committee for Standards in Haematology. Goldman J. Recommendations for the management of BCR-ABL positive Chronic Myeloid Leukaemia. URL; http://www.bcshguidelines.com/pdf/CML_guidelines_270707.pdf (accessed October 2012).



ACUTE MYELOID LEUKAEMIA (AML) CYTOGENETICS

Introduction

An abnormal karyotype is found in approximately 55% of AML cases at presentation. A large number of chromosome abnormalities have been identified that can aid the diagnosis of AML and identify subtypes; the WHO classification now defines certain groups by their cytogenetic abnormalities, such as t(8;21), inv(16), t(15;17), t(9;11), t(6;9), inv(3) and t(1;22)¹. Cytogenetics can also help to define AML with myelodysplasia-related changes and therapy-related myeloid neoplasms. Importantly, many cytogenetic abnormalities have prognostic implications and are therefore used in risk-stratified treatment regimes^{2,3}.

Good prognosis	t(15;17), t(8;21) and inv16/t(16;16)
Intermediate prognosis	Entities not classified as favourable or adverse including; +8 and others. t(9;11) and t(11;19) MLL translocations
Poor prognosis	Complex karyotype (≥ 4 abnormalities) abn(3q) including inv(3)/t(3;3) -5, del(5q) or add(5q) -7, add(7q)/del(7q) 11q23 (MLL) translocations t(6;11) or t(10;11) t(9;22) -17 or abn(17p)

Adapted from Grimwade et al 2010²

Different prognostic scoring systems may vary (e.g. European LeukemiaNet³) and the significance of cytogenetics will also depend on the demographics of the study population, such as paediatric and elderly cases⁴. A newly defined entity, "monosomal karyotype" (2 or more autosomal monosomies or 1 monosomy with structural abnormalities) is common in elderly patients and, although not distinguished in UK data², has been reported to confer a dismal prognosis⁵. Such prognostic classifications are used to assign risk group in UK MRC AML trials, prospectively in AML17.

All patients should have conventional cytogenetics performed at diagnosis, supplemented by FISH tests as appropriate, to identify favourable and unfavourable prognostic abnormalities^{6,7}. Cytogenetics can be used to monitor response to treatment and can confirm relapse or indicate disease progression.

Referrals

- Full karyotype at diagnosis
- Rapid FISH for t(15;17) at diagnosis (usually <24 hours) in APL
- FISH at diagnosis to detect t(8;21) [RUNX1-RUNX1T1], inv(16) [CBFB], 11q23 rearrangements [MLL] depending on morphology or as requested
- FISH for 5q, 7q, and 17p [TP53] deletions and monosomy 5 and 7 on failed samples
- Post-treatment bone marrows screened for previous abnormality by routine cytogenetics or FISH, as appropriate
- Full karyotype at possible relapse for recurrence of previous abnormality, clonal evolution or new disease.

Technical

Cytogenetic analysis is performed on cultured cells from fresh bone marrow. 20 cells will be fully analysed, according to standard procedures and best practice. Cells may contain cryptic abnormalities and minor clones not represented in the cultured cells, which microscopic analysis may not detect.



Sample requirements

Bone marrow aspirate or peripheral blood specimens should be collected fresh into the supplied transport bottles or alternatively into other lithium heparin-containing tubes. Samples should be sent to the laboratory as soon as possible, preferably on the day of collection.

Summary of services and reporting times

Test	Target Reporting Time (Calendar days)
Rapid FISH at diagnosis	3 working days
Cytogenetic analysis (karyotype)	7
Cytogenetic analysis and/or FISH post-treatment	21
Cytogenetic analysis and/or FISH at relapse or transformation	14

Further Information

The Oncology Cytogenetics laboratory is accredited by Clinical Pathology UK Ltd (ref. no. 2034). CPA standards are compliant with ISO 15189. The laboratory also complies with professional standards issued by the Association for Clinical Cytogenetics (ACC) and participates in all appropriate EQA schemes (UKNEQAS). Please see relevant sections of the Oncology Cytogenetics User Guide for full details.

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MYELODYSPLASTIC SYNDROMES (MDS) CYTOGENETICS

Introduction

Approximately 50% of confirmed, de novo MDS cases have cytogenetic abnormalities at diagnosis, which helps to confirm the presence of a clonal disorder and aids the distinction between MDS and reactive causes of dysplasia. Numerous abnormalities have been described, the most common of which are trisomy 8 (10%), monosomy 7/deletion of 7q (10%), monosomy 5/deletion of 5q (10%), deletion of 20q (5-8%), -Y (5%) and deletions of 17p (3-5%). Some cytogenetic abnormalities, in the context of cytopenias of undetermined origin, can be considered presumptive evidence of MDS in the absence of definitive morphologic features¹. However, none are specific to MDS and are found in AML and other myeloid neoplasms. There are also no specific markers for MDS subgroups, although isolated deletion of 5q, with typical morphology, defines a WHO subtype. There are no specific abnormalities which would confirm transformation to acute leukaemia but a complex or evolving karyotype might be suggestive. Some chromosome abnormalities indicate more aggressive disease and therefore have major prognostic value. Karyotype is therefore included as a criterion in prognostic schemes, together with blast cell proportion and number of cytopenias e.g. International Prognostic Scoring System (see Table below)². Cytogenetics is recommended in all cases where bone marrow examination is indicated^{3,4}.

Table; Cytogenetic risk groups in IPSS for MDS (Greenberg et al, 2012)²

Very good prognosis	-Y, del(11q)
Good prognosis	Normal karyotype, del(5q), del(12p), del(20q). double including del(5q)
Intermediate prognosis	del(7q), +8, +19, i(17q), any other single or double independent clones
Poor prognosis	-7, inv(3)/t(3q)/del(3q), double including -7/del(7q), Complex karyotype (= 3 abnormalities)
Very poor prognosis	Complex karyotype (>3 abnormalities)

Recently developed new therapeutic agents (such as 5-azacytidine and lenalidomide) have been used for the treatment of MDS and have been reported to be effective in specific cytogenetic subgroups^{5,6}.

Referrals

Samples are accepted on all cases of suspected MDS where bone marrow examination is indicated. Full cytogenetic analysis will be performed to test for clonal abnormalities of prognostic significance. Samples that fail to grow in culture will have FISH for 5q and 7q deletions.

Patients with macrocytic anaemia and other cytopenias are often referred for investigation of possible MDS. It would be helpful if cytogenetics requests were called off (to avoid unnecessary analyses) if the diagnosis of MDS is excluded or cytogenetics is no longer required after the examination of bone marrow morphology.

Periodic re-investigation of bone marrows from untreated MDS patients may be warranted, particularly if there is change in clinical or laboratory findings.

After treatment, a screen for previous cytogenetic abnormalities can be performed to monitor remission, if a cytogenetic marker was present at diagnosis.



Technical

Cytogenetic analysis is performed on cultured cells from fresh bone marrow. The karyotype from a minimum of 20 cells will be fully analysed, according to standard procedures and best practice guidelines. Cells may contain cryptic abnormalities and minor clones may be present that are not represented in the cultured cells, which microscopic analysis may not detect.

Sample requirements

Bone marrow aspirate specimens should be collected fresh into the supplied transport bottles or alternatively into other lithium heparin-containing tubes. Samples should be sent to the laboratory as soon as possible, preferably on the day of collection. Peripheral blood specimens from typical MDS cases are unlikely to be useful.

Summary of services and reporting times

Test	Target Reporting Time (Calendar days)
Cytogenetic analysis (karyotype) of new MDS	21
FISH on the above	21
Cytogenetic analysis of MDS ?transforming to AML	7
Cytogenetic analysis of ?secondary MDS	14

Further Information

The Oncology Cytogenetics laboratory is accredited by Clinical Pathology UK Ltd (ref. no. 2034). CPA standards are compliant with ISO 15189. The laboratory also complies with professional standards issued by the Association for Clinical Cytogenetics (ACC) and participates in all appropriate EQA schemes (UKNEQAS). Please see relevant sections of the Oncology Cytogenetics User Guide for full details.

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1. Swerdlow, S. H., E. Campo, N. L. Harris, E. S. Jaffe, S. A. Pileri, H. Stein, J. Thiele, J. W. Vardiman, Eds. (2008). *WHO Classification of tumours of haematopoietic and lymphoid tissues*. World Health Organization Classification of Tumours. Lyon, IARC.
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APLASTIC ANAEMIA (AA) CYTOGENETICS

Up to 15% of cases of aplastic anaemia exhibit clonal chromosomal abnormalities which may aid the distinction of AA from hypoplastic MDS, although there is no consensus that the presence of chromosomal abnormalities is incompatible with a diagnosis of AA and therefore confirms MDS¹. The finding of a cytogenetic abnormality in AA can be a strong indication of clonal evolution to MDS, which is important to characterise as the prognosis of these patients is less favourable and treatment choices differ, in particular when high-risk chromosomal abnormalities are involved. The most common cytogenetics abnormalities in AA are trisomy 8 and aberrations of chromosome 7. Cases with abnormal cytogenetics, particularly monosomy 7 and complex karyotypes, have been reported to show an increased rate of transformation to AML and a poor response to immunosuppressive therapy²⁻⁴.

Technical

Cytogenetic analysis is performed on cultured cells from fresh bone marrow. The karyotype from a minimum of 20 cells will be fully analysed, according to standard procedures and best practice guidelines. Cells may contain cryptic abnormalities and minor clones may be present that are not represented in the cultured cells, which microscopic analysis may not detect.

Sample requirements

Bone marrow aspirate specimens should be collected fresh into the supplied transport bottles or alternatively into other lithium heparin-containing tubes. Samples should be sent to the laboratory as soon as possible, preferably on the day of collection. Low cellularity of the aspirates in AA often leads to small numbers of mitotic cells to analyse and therefore unsuccessful cytogenetic testing. Peripheral blood specimens from typical AA cases are unlikely to be useful.

Summary of services and reporting times

Test	Target Reporting Time (Calendar days)
Cytogenetic analysis (karyotype) of AA	21
FISH on the above	21
Cytogenetic analysis of AA ?transforming to MDS	14

Further Information

The Oncology Cytogenetics laboratory is accredited by Clinical Pathology UK Ltd (ref. no. 2034). CPA standards are compliant with ISO 15189. The laboratory also complies with professional standards issued by the Association for Clinical Cytogenetics (ACC) and participates in all appropriate EQA schemes (UKNEQAS). Please see relevant sections of the Oncology Cytogenetics User Guide for full details.

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MYELOPROLIFERATIVE NEOPLASMS

MPN are a heterogeneous group of disorders including classical MPN (PRV, ET and IMF) and a number of other entities characterised by proliferation of mature cells of myeloid lineage. By definition, the distinction of MPN from CML requires exclusion of t(9;22)/BCR-ABL1. Ideally, MPN patient pathways should include molecular exclusion of JAK2 V617F for PV, ET, and PMF before referral for cytogenetic analysis.

Polycythaemia Rubra Vera (PRV)

Cytogenetic abnormalities are found in 10~20% of patients overall with PRV; infrequently at diagnosis^{1,2} but increasing in frequency with disease course³. The abnormalities are general myeloid markers including trisomy for chromosomes 8 and 9, del(20q), del(13q) and 1q gain. Generally, the abnormalities have no apparent adverse affect and trisomy 8 and 9 are known to persist in PRV for many years without disease progression. However, an abnormal karyotype, as a marker of clonality, is a major diagnostic criterion^{4,5} and cytogenetics should be performed where absolute erythropoiesis is confirmed in the absence of JAK2 V617F mutation⁶.

Patients who progress to myelofibrosis or MDS/AML almost always have karyotypic abnormalities and there is an increase in frequency of chromosome aberrations and increasing karyotype complexity in disease progression. While there is no specific abnormality that will confirm transformation, abnormalities such as del(5q), monosomy 7, del(17p) or complex changes are suggestive.

Essential Thrombocythaemia (ET)

An abnormal karyotype is unusual in ET (~5% of cases) and the abnormalities, when present, are general myeloid markers (del(20q), +8, +9, del(13q), gain of 1q, del(5q)). JAK2 mutation is present in 50% of patients but the predictive value is poor and there is lack of diagnostic specificity. Given the low abnormality rate, the main utility of cytogenetics is the exclusion of CML by the absence of the t(9;22) or BCR-ABL1. BCR-ABL1 testing should be considered to exclude CML, especially for cases with high risk features (e.g. basophilia, left shifted WBC, granulocyte count $>16 \times 10^9/l$, small megakaryocytes, difficult to control thrombocytosis). A bone marrow biopsy is recommended in a diagnostic algorithm for the differential diagnosis of ET from other myeloid neoplasms; cytogenetics is advised but not mandatory but may be helpful to exclude other abnormalities associated with platelet gain, including 3q26 abnormalities and del(5q)⁴. Again there are no specific chromosome rearrangements that will confirm transformation but abnormalities of chromosomes 7 and 17, 7q-, der(1;7)17p-, i(17q) are suggestive.

Primary Myelofibrosis

Chromosome abnormalities are found in 60% of cases but again are not specific and include del(20q) and del(13q)⁷. Cytogenetic analysis is included in a diagnostic algorithm for myelofibrosis⁴. Cytogenetics is incorporated into the updated prognostic scoring system for Primary Myelofibrosis, DIPSS Plus, and also predicts leukaemia-free survival but should be considered alongside other risk factors of the scheme⁸. There is no specific marker for transformation but a complex karyotype and abnormalities of chromosomes 5, 7 and 17 are again strongly suggestive.

Risk	Cytogenetic Abnormality
Favourable	Normal karyotype or sole abnormalities of 20q-, 13q- or +9 and all other cytogenetic findings
Unfavourable	Complex karyotype (≥ 3 abnormalities), or sole or two +8, -7/7q-, i(17q), -5/5q-, 12p-, inv(3), or 11q23 rearrangement

From Caramazza et al 2011⁹ used in DIPSS+ for myelofibrosis



Chronic Myelomonocytic Leukaemia (CMML)

Cytogenetic abnormalities are found in up to 40% of cases but are rarely specific; the commonest being trisomy 8, monosomy 7 or deletion of 7q and rearrangements of 12p. The rare translocations t(5;12) and t(8;13) appear to define a subset of patients with a specific disease entity including eosinophilia (see below). Distinction between CMML and atypical chronic myeloid leukaemia may be difficult and BCR-ABL1 FISH will be performed on request. A new CMML-specific prognostic scoring system (CPSS) includes cytogenetic risk for stratification and identifies trisomy 8, abnormalities of chromosome 7 and complex karyotype (>3 chromosomal abnormalities) as high risk¹³.

Hypereosinophilia

Rare chromosomal translocations have been described in eosinophilic MPD that define a WHO class (Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of PDGFRA, PDGFRB and FGFR1) that are important because of their apparent clinical utility; diseases with PDGFRA or PDGFRB rearrangements are reported to respond to imatinib. The rare FIP1L1- PDGFRA gene fusion results from an interstitial deletion at chromosome band 4q12 and cannot be detected by routine cytogenetics and must be tested by FISH. The related t(5;12) translocation or variants involving the PDGFRB gene, may be detected by routine cytogenetics, but would also require confirmation by FISH before treatment. Other rearrangements associated with eosinophilia are detected such as FGFR1 at 8p11. Diagnosis of HES requires absence of BCR-ABL1 this is also examined by cytogenetics but can be requested by FISH in cases of high eosinophilia.

Cytogenetics should be performed to support diagnosis of this disease only after reactive cases and a number of other diseases associated with eosinophilia have been excluded⁴. However, persistent unexplained eosinophilia (whether formally diagnosed as HES or not) will receive a full conventional cytogenetic analysis and will be screened for the FIP1L1-PDGFRB fusion by FISH.

Systemic Mastocytosis

A full cytogenetic analysis is performed although specific chromosome abnormalities are not recognised. Screening for KIT D816V mutation is not available in this laboratory but advice will be provided on where to forward the sample for molecular testing, which will require separate sample bottles. Only cases reported to have eosinophilia will be screened for FIP1L1-PDGFRB fusion by FISH (see CEL/HES above).

Referrals

1. Full karyotype (when reactive causes of myeloproliferation is excluded)
2. FISH for BCR-ABL1 performed on all cases of ET. Full karyotype of confirmed cases of ET by special request. BCR-ABL1 FISH on other cases of MPN by request
3. FISH for FIP1L1-PDGFRB in cases of persistent eosinophilia. FISH to confirm PDGFRB (5q) and FGFR1 (8p11) if indicated.
4. JAK2 V617F mutation screening is NOT available in this department

Technical

Cytogenetic analysis is performed on cultured cells from fresh bone marrow. The karyotype from a minimum of 20 cells will be fully analysed, according to standard procedures and best practice guidelines. Cells may contain cryptic abnormalities and minor clones may be present that are not represented in the cultured cells, which microscopic analysis may not detect.

Sample requirements

Bone marrow aspirate specimens should be collected fresh into the supplied transport bottles or alternatively into other lithium heparin-containing tubes. Samples should be sent to the laboratory as soon as possible, preferably on the day of collection. Peripheral blood specimens from typical MPN cases may be useful if immature cells are present. A fresh bone marrow trephine specimen is an option, if the marrow is fibrotic or otherwise difficult to aspirate.



Summary of services and reporting times

Test	Target Reporting Time (Calendar days)
Cytogenetic analysis (karyotype)	21
FISH	21
Cytogenetic analysis of MPN ?transforming to AML	14

Further Information

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LYMPHOBLASTIC LEUKAEMIA / LYMPHOMA

Cytogenetic abnormalities are found in the majority of B-lymphoblastic leukaemia/lymphoma by conventional cytogenetic analysis supplemented by FISH. WHO recognises a number of subtypes with recurrent genetic abnormalities which define specific entities with distinct phenotypic and prognostic features, including t(9;22) [BCR-ABL1], 11q23 [MLL], t(12;21) [ETV6-RUNX1], Hyperdiploidy, Hypodiploidy/Haploidy, and t(1;19) [TCF3-PBX1]¹. The frequency and significance of abnormalities differs between adult and paediatric patients (see table below)^{2,3}. The translocation, t(9;22), giving rise to the Ph chromosome, is more common in adults than children, is associated with a poor prognosis and imatinib is incorporated into intensive treatment regimes⁴. Translocations involving the MLL gene, such as t(4;11), are common, especially in infants and are associated with an unfavourable prognosis. The t(12;21) is the most common translocation in paediatric cases and is only detectable by FISH for the ETV6-RUNX1 gene fusion and is associated with good overall survival. FISH for this translocation also detects amplification of RUNX1 gene (iAMP21) which is incorporated into trial protocols as a high risk abnormality.⁶ The department provides cytogenetic and FISH investigations to support UKALL14 and UKALL2011 trials.

	Childhood ALL*	Adult ALL**
Good prognosis	t(12;21) [ETV6-RUNX1], high hyperdiploidy	high hyperdiploidy, del(9p)
Intermediate prognosis	Other abnormalities including; t(1;19)(q23;p13) dup(1q) del(6q) -7 Abnormal 9p dic(9;20)(p13;q11) dic(9;12)(p11-21;p11-13) Abnormal 11q	Other abnormalities including; t(1;19)(q23;p13) del(6q) -7 +8 11q23 [MLL] translocations other than t(4;11) Abnormal 11q del(12p) Loss of 13q Abnormal 17p
Poor prognosis	t(9;22) 11q23 [MLL] translocations iAMP21 [RUNX1 amplification] Near haploidy (<30 chromosomes) Low hypodiploidy (30-39 chromosomes) t(17;19)(q23;p13) Abnormal 17p Loss of 13q	t(9;22) t(4;11) Low hypodiploidy (30-39 chromosomes)/ near triploidy Complex karyotype (≥5 abnormalities)

Adapted from; *Moorman et al 2010

** Moorman et al 2007

Services Offered

Cytogenetics should be carried out at diagnosis in all cases. Cytogenetic abnormalities can be used to monitor response to therapy and confirm relapse.

- Full karyotype at diagnosis
- Rapid FISH for BCR-ABL1 and MLL gene rearrangements (and ETV6-RUNX1 in children)
- Subsequent, sequential FISH at diagnosis for TCF3-PBX1, specific MLL translocations, Hyperdiploidy and Hypodiploidy, as required



- Post-treatment bone marrows screened for previous abnormality by routine cytogenetics or FISH, as appropriate
- Full karyotype and relevant FISH at possible relapse

Technical

Cytogenetic analysis is performed on cultured cells from fresh bone marrow. 20 cells will be fully analysed, according to standard procedures and best practice. Cells may contain cryptic abnormalities and minor clones not represented in the cultured cells, which microscopic analysis may not detect. ALL disease cells are notorious for their poor survival in vitro and rapid transport to the laboratory is critical for successful chromosome analysis and FISH. ALL disease cells also often have poor chromosome morphology. The sensitivity of monitoring follow-up cases in remission is therefore likely to be severely limited.

Sample requirements

Bone marrow aspirate specimens should be collected fresh into the supplied transport bottles or alternatively into other lithium heparin-containing tubes. Samples should be sent to the laboratory as soon as possible, preferably on the day of collection. Peripheral blood specimens with disease cell involvement are suitable specimens for diagnosis.

Summary of services and reporting times

Test	Target Reporting Time (Calendar days)
Rapid FISH at diagnosis	3 working days
Cytogenetic analysis (karyotype)	7
Subsequent FISH prognostic markers	14
Cytogenetic analysis and/or FISH post-treatment	21
Cytogenetic analysis and/or FISH at ?relapse or transformation	14

Further Information

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References

1. Swerdlow, S. H., E. Campo, N. L. Harris, E. S. Jaffe, S. A. Pileri, H. Stein, J. Thiele, J. W. Vardiman, Eds. (2008). *WHO Classification of tumours of haematopoietic and lymphoid tissues*. World Health Organization Classification of Tumours. Lyon, IARC.
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LYMPHOMA

Chromosome translocations help to define disease subtypes, the most common are the t(11;14) associated with mantle cell lymphoma, t(14;18) with follicular lymphoma and t(8;14) with Burkitt lymphoma. However, these rearrangements are not specific to one disease and the profile of chromosome translocations needs to be interpreted alongside morphology and immunophenotype for accurate diagnosis. Other translocations such as t(11;18) in MALT lymphoma and those involving the ALK gene at 2p23 in Anaplastic Large Cell Lymphoma can be useful diagnostically.

Furthermore, t(14;18) (IGH@/BCL2), t(8;14) (IGH@/MYC), 3q27 (BCL6), 17p (TP53) deletion, are informative prognostic markers in certain situations and predict progression to high grade disease.

FISH is available for all of the above abnormalities and is the test of choice as mature lymphocytic cells can be difficult to grow in culture. FISH on paraffin-embedded tissue sections is usually the most practical as fresh tissue is not usually available (see [FISH ON PARAFFIN-EMBEDDED TISSUE FOR SOLID TUMOURS/LYMPHOMA](#) page 44).

Cytogenetics can be performed on lymph nodes providing fresh material is sent in tissue culture medium to the Cytogenetics Laboratory, as soon as possible. Alternatively peripheral blood or bone marrow can be used as test material if these are involved. However, the limited number of cells, which can be fully analysed by routine cytogenetic analysis, makes this test of little value in staging. Bone marrow infiltration should be confirmed by other methods and significant involvement should be confirmed before cytogenetic analysis is performed.

Services Offered

- FISH on paraffin embedded tissue sections (see page 44)
- FISH or full karyotype on fresh primary tissue
- FISH or full karyotype on bone marrow or blood, only if involved (lymphocytosis present) or if FISH abnormal on other tissue
- In [Burkitt Lymphoma](#); rapid FISH for MYC and IGH@-MYC followed by FISH for BCL6 and IGH@-BCL2 to aid the differential diagnosis of BL from DLBCL. Full karyotype on bone marrow or blood, if involved to examine additional abnormality and karyotype complexity.

Summary of services and reporting times

Test	Target Reporting Time (Calendar days)
Rapid MYC FISH at diagnosis of ?Burkitt	3 working days
Cytogenetic analysis (karyotype) and subsequent FISH in ?Burkitt	7
Cytogenetic analysis and/or FISH for other lymphoma subtypes	21

Further Information

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CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL)/ SMALL LYMPHOCYTIC LYMPHOMA

Introduction

Cytogenetic abnormalities can be found in the majority of patients with CLL although, at this time, most do not provide useful diagnostic information. FISH identifies genomic abnormalities in approximately 80% of newly diagnosed cases, including trisomy 12 and deletions of 11q, 13q, and 17p which have been shown to have prognostic significance.¹ However only 11q (ATM gene) and 17p (TP53 gene) deletions are associated with shortened survival and are used to guide treatment decisions in individual patients². These are therefore recommended in clinical guidelines.^{2,3,4}

17p/TP53 deletions occur in less than 5% of newly diagnosed patients but increase in frequency with advancing disease. TP53 gene deletion by FISH predicts a very poor prognosis, and resistance to conventional chemotherapy but alternative treatments, such as alemtuzumab, may be effective⁵. Deletion of 11q/ATM also show a poor prognosis in some studies and resistance to chemotherapy but there are some reports that the inclusion of rituximab in combination with FC chemotherapy may be beneficial^{6,7}.

FISH for TP53 and ATM deletions is valid in SLL, although the test tissue must be involved. FISH for deletion is possible on paraffin-embedded tissue sections but the tissue must be heavily infiltrated with disease cells, due to the high false positive background (see below).

FISH studies are useful in differentiating mantle cell lymphoma from CLL. However, blanket FISH testing for IGH@-CCND1 for the exclusion of mantle cell lymphoma is not performed. Please request IGH@/CCND1 FISH for t(11;14) in all cases of CLL with atypical morphology or a CLL immunophenotype score of 3 or less⁸.

Referrals

There is no evidence that treatment of early CLL improves overall outcome and so FISH testing is recommended only prior to first treatment. Chromosomal abnormalities may develop during disease course and FISH analysis should also be considered prior to subsequent treatments.

FISH tests are not suitable for monitoring remission. Referred samples must be from involved tissue with significant lymphocytosis. Peripheral blood is suitable test material, in most cases. CLL patients suspected of secondary myeloid disease need to be highlighted as they will be handled differently.

Technical

FISH for TP53 and ATM deletions is a standardised procedure and the test of choice. FISH for trisomy 12 and 6q and 13q deletions are available on request.

Cell culture of mature lymphocytes in CLL is unreliable and karyotyping is therefore difficult. Full karyotyping is not routinely performed, but is available, by special advance request.

FISH for deletion has a high false positive background and levels of less than 10% for TP53 deletion and less than 5% for ATM deletion are not considered significant and will not be reported⁹. Levels within 5% above these cut-offs will be considered borderline and reported suggesting a need to confirm the result in a later sample (e.g. ATM 5~10% and TP53 10~15%).

Sample requirements

Bone marrow aspirate or peripheral blood specimens should be collected fresh into the supplied transport bottles or alternatively into other lithium heparin-containing tubes. Samples should be sent to the laboratory as soon as possible, preferably on the day of collection.



Summary of services and reporting times

Test	Target Reporting Time (Calendar days)
FISH for 17p/TP53 and 11q/ATM deletions on peripheral blood or bone marrow on all cases referred. Cell culture and storage of bone marrow cells in case conventional cytogenetic analysis indicated at a later date	7
FISH for t(11;14) [IGH@-CCND1] in atypical cases by request/indication	7 days, if indicated
Full FISH panel including 13q deletion and trisomy 12 (as well as ATM and TP53)	Not routine. By special request only
Conventional cytogenetic analysis	Not routine. Please enquire

Further Information

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MYELOMA CYTOGENETICS

Introduction

Myeloma shows a range of recognised cytogenetic abnormalities which can aid subclassification of the disease and are strong prognostic factors¹. The IMWG recommends a basic strategy for FISH testing for t(4;14), t(14;16) and TP53 deletion, due to their strong negative impact on prognosis, which is also recommended in BCSH Guidelines¹⁻³. The combination of FISH testing and ISS (International Staging System) has been shown to improve risk assessment in myeloma^{4,5}. An extended FISH panel, also including tests for 1q21 gain and t(14;20), showed a cumulative effect on prognosis and defined a high risk group with 2 or more abnormalities⁴.

There is emerging evidence of the role of cytogenetic abnormalities defined by FISH studies in directing specific therapies but this is under evaluation.

Conventional cytogenetic analysis is hampered by the low proliferation rate of disease cells in culture and a number of the abnormalities being cryptic. Monosomy 13, as detected by conventional karyotyping, has been reported not to be an independent prognostic marker, which largely negates the value of routine conventional karyotyping⁶. However, this may still have some prognostic relevance².

Neutral prognosis	t(11;14), t(6;14). Hyperdiploidy
Poor prognosis	t(4;14); IGH@-FGFR3 t(14;16); IGH@-MAF t(14;20); IGH@-MAFB TP53 deletion Gain of 1q21

If a patient already has an identified high-risk feature at diagnosis, then there is no need to perform repeat investigations at relapse. However, if a patient is in a low-risk group, a full panel of FISH tests will be performed at relapse, for risk re-stratification, to look for new emerging clones that were not detected at diagnosis².

Referrals

The FISH service tests for prognostic markers at diagnosis or relapse. Analysis is applicable for symptomatic cases of confirmed myeloma only. Cases with an uncertain diagnosis will be stored until a diagnosis of myeloma is confirmed, so that unnecessary tests are not performed on MGUS, in which the abnormalities do not have the same significance. Please contact the laboratory to activate a case following diagnosis.

FISH testing is not suitable to monitor disease course and remission samples cannot be tested. Myeloma patients suspected of secondary disease need to be highlighted as they will be handled differently.

Technical

Where myeloma is indicated on the referral card and sufficient cells are available, FISH is performed on CD138 separated cells and so disease plasma cells should be enriched in the sample. Rare CD138 negative cases will not be enhanced. To allow time for cell separation and sample processing all samples should be received before 2pm on Friday.



Sample requirements

Bone marrow aspirate specimens should be collected fresh into the supplied transport bottles or alternatively into other lithium heparin-containing tubes. Samples should be sent to the laboratory as soon as possible, preferably on the day of collection.

Summary of services and reporting times

Test	Target Reporting Time (Calendar days)
Basic 3 FISH panel for IGH@-FGRF3, IGH@-MAF, TP53 deletion. Cell culture and storage of bone marrow cells in case conventional cytogenetic analysis indicated	21
5 FISH panel including 1q21 and IGH@-MAFB	Not routine. Please enquire
Conventional cytogenetic analysis	Not routine. Please enquire

Further Information

The Oncology Cytogenetics laboratory is accredited by Clinical Pathology UK Ltd (ref. no. 2034). CPA standards are compliant with ISO 15189. The laboratory also complies with professional standards issued by the Association for Clinical Cytogenetics (ACC) and participates in all appropriate EQA schemes (UKNEQAS). Please see relevant sections of the Oncology Cytogenetics User Guide for full details.

References

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FISH ON PARAFFIN-EMBEDDED TISSUE (PET) FOR SOLID TUMOURS

The following FISH services are offered on PETs:

- HER2 in breast carcinoma
- Lymphoma diagnosis
- Brain tumour gliomas
- Sarcoma diagnosis
- ALK gene rearrangement in non-small cell lung carcinoma
- Malignant mesothelioma

Sample requirements

- The laboratory only accepts tissue sections. The optimal thickness for all sections is ~3µm. The laboratory currently does not accept uncut blocks of tissue, and these will be returned to the sender.
- Sections should be mounted on APES-coated (or equivalent) slides. Please label all slides clearly with AT LEAST TWO unique patient identifiers, e.g. name and pathology no.
- Send one slide per FISH test requested, plus a spare slide. Please refer to the table below with regards to number of slides for a specific referral reason.
- In cases where only part of the tissue is infiltrated, or only part of the tissue is appropriate for screening, please provide an H&E with the relevant area marked.
- Archival material is accepted.
- Send all slides in a protective container together with the referral card and preferably your own Histopathology report. All required fields on the Oncology Cytogenetics laboratory referral card are coloured in blue (see page 5).
- Please address all samples to Oncology Cytogenetics (see page 2).

Referral	Minimum number of slides required (plus one spare)
Burkitt lymphoma	4 + 1
Mantle cell lymphoma	1 + 1
Follicular lymphoma	1 + 1
MALT	1 + 1
Brain tumour glioma	2 + 1
HER2	5 + 1
ALK	1 + 1
Mesothelioma	1 + 1



HER2 in Breast Cancer

HER2 FISH

The laboratory provides an accurate and timely HER2 FISH service, to determine HER2 status for the use of adjuvant trastuzumab (Herceptin®) therapy, in support of NICE guidance in early breast cancer¹. In conjunction with the Breast Tumour Receptor section of the Histopathology department 496 HER2 FISH were performed in 2011. We follow current HER2 testing guidelines², which recommend a two-tier service utilising immunohistochemistry to detect HER2 protein expression with analysis of equivocal HER2 (2+) cases by FISH to detect gene amplification. However there is provision in the current guidelines for the use of FISH as a stand-alone frontline test. Other probes such as TOP2A, are available by special request. The laboratory is the largest and most experienced HER2 FISH service for the Greater Manchester and Cheshire Cancer Network and receives specimens from several HER2 immunochemistry testing centres for FISH.

HER2 testing for gastric cancer

Herceptin® therapy has also been shown to be effective in HER2 positive patients with advanced gastric or gastroesophageal cancer (ToGA study)³. In November 2010 NICE approved the use of Herceptin for patients with HER2 positive (HER2 3+ by immunohistochemistry) gastric or gastroesophageal cancer⁴. Following successful validation of HER2 in gastric specimens in 2010, the laboratory now routinely performs HER2 testing in gastric specimens, in accordance with Ruschhoff's recommendations⁵. It is recommended that all cases of gastric tumours are tested and referred directly from the pathologist to avoid delay and avoid possible problems of storage. Further information on arrangements for HER2 testing can be obtained from Dr Mansoor at was.mansoor@christie.nhs.uk.

HER2 FISH is performed using approved dual colour assays, comprising two fluorescent-labelled probes for the HER2 gene and an internal control of chromosome 17 centromere. The system detects variation in chromosome copy number (polysomy and monosomy). Results are expressed as a ratio of HER2 copy number relative to Chromosome 17 copy number. HER2 amplification of invasive cancer is confirmed with a HER2/Ch17 ratio of greater than 2.00. Borderline cases with HER2/Ch 17 ratios between 1.80 and 2.20 will undergo appropriate additional work-up. Cases achieving a ratio of between 1.80 and 1.99 will be reported as Borderline not amplified and considered HER2 negative, those with a ratio 2.00 to 2.20 will be reported Borderline amplified and considered HER2 positive to aid clinical management.

Breast Tumour Receptors

The Breast Tumour Receptor laboratory is an expert specialised service which has been performing HER2 IHC and other breast receptors (including ER, PgR, Ki67, EGFR and Androgen Receptor) in paraffin embedded tissues since 1996. The HER2 FISH service was launched in 2001 to complement the HER2 IHC service. Using validated and standardised methods the laboratory performed 1900 routine HER2 IHC tests and 496 HER2 FISH tests in 2011. These numbers are significantly in excess of the minimum requirement (250 IHC & 100 FISH) to support quality assured testing².



HER2 IHC interpretation: Any given case showing 30% of invasive cancer cells expressing complete, strong membrane staining is regarded as HER2 positive. Cases expressing moderate membrane staining in greater than 10% of invasive cells, or cases with less than 30% invasive cells expressing strong complete membrane staining are considered HER2 2+ or 2+/3+ and forwarded for assessment by FISH.

Assay validation: The laboratory conducts a continuous audit evaluating concordance levels between HER2 IHC and FISH. During the three years (2009-2011) 1586 cases have been tested in parallel for HER2 protein expression by IHC and HER2 gene amplification by FISH with the following results:

HER 2 protein (IHC)	HER2 FISH result
Negative or 1+ (n = 238)	96% FISH Negative, 1% FISH Borderline, 3% FISH Amplified
2+ (n = 1072)	11% FISH Amplified, 5.5% FISH Borderline, 83% FISH Negative
2+/3+ (n = 229)	66% FISH Amplified, 11% FISH Borderline, 23% FISH Negative
3+ (n = 101)	94% FISH Amplified, 4% FISH Borderline, 2% FISH Negative

Summary of HER2 status

The sample population received at The Christie between 2009 and 2011 were primarily new breast cancers including screen-detected lesions. In our hands 80% of routinely received breast cancers are ER positive. There is a strong relationship between ER negativity and HER2 positivity. Up to a third of tumours determined ER negative are HER2 positive whereas only approximately 5% of ER positive tumours are also HER2 positive. Our combined ER/HER2 positivity rates for all tumours received for dual testing between 2009 and 2011 are as follows

ER Negative Tumours

HER2 status	Overall Frequency
Negative	7.5%
1+	3.1%
2+	3.8%
3+	6.5%

ER Positive Tumours

HER2 Status	Overall Frequency
Negative	27.4%
1+	27%
2+	19.3%
3+	5.2%



For the period Jan 2011 – Jun 2012

Total samples 2+ or 2+/3+ demonstrating HER2 FISH Amplification: 27% *

Total samples HER2 Positive (i.e. HER2 3+ & HER2 FISH Positive): 12%

* Please note that this figure is a reflection of all samples received by The Christie laboratory as well as those from external HER2 testing centres. It is not therefore a reflection of the current rate of HER2 2+ equivocal cases in the region.

Turnaround Times

Turnaround times for HER2 IHC and FISH are good, the limiting factor in producing optimal turnaround efficiency, from biopsy to authorised report, is dependent on the time taken for the sample to reach the laboratory from the requestor. Turnaround times are assessed as time of receipt in the laboratory to release of report for HER2 IHC & HER2 FISH.

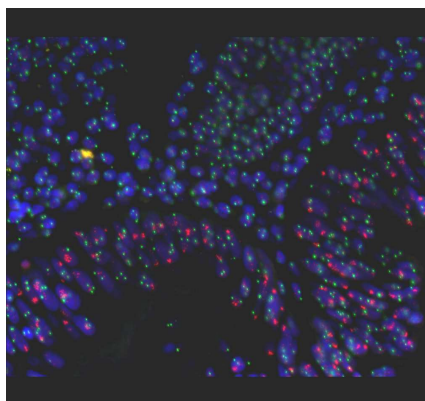
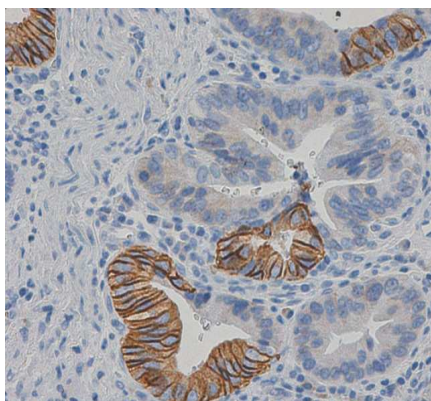
During 2011 the average monthly turnaround time for HER2 by IHC was 3.3 days

Average turnaround time for HER2 FISH was 5.3 days.

Providing the sample is received by the laboratory in a timely manner, service users can expect to receive their results from biopsy within one week for IHC and two weeks for FISH, ensuring results are available for MDT meetings.

References

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HER-2 Positive Gastric Carcinoma (IHC & FISH) showing the heterogeneous nature of the disease
(images from The Christie, Histopathology / Oncology Cytogenetics Departments)



Lymphoma Diagnosis

FISH on paraffin embedded tissue (PET) for lymphoma diagnosis is offered as part of Greater Manchester & Cheshire Cancer Network Haematology Malignancy Diagnostics (see pg. 27) and is accessed by referral of cases for specialist review or opinion by the Histopathology department at the Christie. Cases referred directly for FISH are accepted. Please also see Oncology Cytogenetics User Guide (pg 29) for referrals of fresh samples for lymphoma diagnosis.

Burkitt lymphoma

The t(8;14) translocation involving rearrangement of the MYC gene with IGH@ is observed in ~ 90% of Burkitt lymphoma, with the IGK@ and IGL@ variants, t(2;8) and t(8;22) accounting for the remainder. Confirmation of one of these translocations, such as by FISH with IGH@/MYC and MYC gene probes, is considered essential for an accurate diagnosis. Morphologically atypical Burkitt lymphoma cases are generally also associated with a rearrangement of the MYC gene, as are a small proportion of high grade follicular lymphomas and diffuse large-B-cell lymphoma. However, the distinction between Burkitt and DLBCL is critical because these two types of lymphoma require different treatments; relatively low-dose chemotherapy regimens are typically used to treat DLBCL but they are inadequate for Burkitt lymphoma, which is highly sensitive to intensive chemotherapy regimens. All Burkitt referrals will also be tested with IGH@/BCL2 and BCL6 probes to aid this distinction; Burkitt is associated with simple karyotypes (if a full cytogenetic result is available) and absence of t(14;18) and BCL6 gene rearrangements. Further, rapid diagnosis of this highly proliferative tumour is essential for optimal prognosis and most appropriate management.

Service offered

- Rapid FISH diagnosis of MYC gene rearrangements
- IGH@/MYC dual fusion to detect the t(8;14) translocation
- MYC breakapart to detect variant MYC translocations
- IGK@ and IGL@ detect t(2;8) and t(8;22) immunoglobulin gene variants
- IGH@/BCL2 to detect the t(14;18)
- BCL6 to detect rearrangements of 3q27

Follicular lymphoma

70-80% of follicular lymphoma cases show the t(14;18) translocation, or the rarer IGK and IGL variants, t(2;18) and t(18;22), all involving rearrangement of BCL2 on 18q21. A small proportion of transforming follicular lymphomas will also have a BCL6 or MYC rearrangement.

Service offered

- IGH@/BCL2 to detect the t(14;18) translocation
- BCL2 breakapart, BCL6 and MYC breakapart upon request only



Mantle cell lymphoma

70-75% of mantle cell lymphomas have the t(11;14) translocation involving the CCND1 locus. A MYC or BCL2 rearrangement is typically absent.

Service offered

- IGH@/CCND1 dual fusion to detect the (11;14) translocation

Other services offered

- MALT1 breakapart to detect rearrangements of 18q21 seen in extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma).
- ALK breakapart to detect rearrangements of 2p23, including the t(2;5) translocation associated with anaplastic large cell lymphoma



Brain tumour gliomas

Co-deletion of 1p and 19q has been shown to be a statistically significant predictor of improved overall survival and chemosensitivity in patients with oligodendroglioma and anaplastic oligodendroglioma. Furthermore, there is a strong association between 1p/19q co-deletion and the pure oligodendroglial phenotype, although this genetic alteration can be seen more rarely in other glioma types.

We offer a FISH screen to establish 1p/19q deletion status on patients with oligodendroglioma and oligoastrocytic tumours. EGFR amplification in higher grade tumours can be provided upon request.

Oncology Cytogenetics were one of the first participants in the pilot EQA for these tests in neurological tumours in 2012.

References

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2. Smith JS, Perry A, Borell TJ, Lee HK, O'Fallon J, Hosek SM, Kimmel D, Yates A, Burger PC, Scheithauer BW, Jenkins RB. Alterations of chromosome arms 1p and 19q as predictors of survival in oligodendrogliomas, astrocytomas, and mixed oligoastrocytomas. *J Clin Oncol*. 2000 Feb;18(3):636-45.



Sarcoma

We currently provide a service for soft tissue sarcomas on PETs using commercially available FISH probes.

Probes available;

- EWSR1 to detect rearrangements of 22q12, including the t(11;22) translocation and variants seen in Ewing's sarcoma/PNET, t(11;22) in desmoplastic small round cell tumour, t(10;22) clear cell sarcoma, t(9;22) in chondrosarcoma and other tumour types, to aid classification.
- SS18 (formerly SYT) to detect rearrangements of 18q11.2 found in the t(X;18) translocation in synovial sarcoma.
- DDIT3 (formerly CHOP) breakapart probe to detect rearrangements of 12q13 including t(12;16) seen in myxoid/round cell liposarcomas.
- MDM2 (with control) for evaluation of MDM2 amplification status to distinguish atypical lipomatous tumour, well-differentiated liposarcoma or dedifferentiated liposarcoma from benign lesions. MDM2 amplification status has been defined in some studies as MDM2 to control probe ratio of greater than 2 (Weaver et al, (2008)), although no standardised scoring criteria currently exist.
- FOXO1 to detect t(2;13) and t(1;13) in Alveolar Rhabdomyosarcoma
- COL1A1-PDGFB fusion in Dermatofibrosarcoma protuberans

FISH for some of the less common tumours can be undertaken with prior agreement with the laboratory. Please contact the Head of Department to discuss the details of specific cases.

Weaver J, Downs-Kelly E, Goldblum JR, Turner S, Kulkarni S, R Tubbs RR, Rubin BP, Skacel M. Fluorescence in situ hybridization for MDM2 gene amplification as a diagnostic tool in lipomatous neoplasms. *Modern Pathology*. 2008 (21): 943-949



ALK rearrangement in non-small cell lung cancer

Approximately 3~7% of cases of non-small cell lung cancer (NSCLC) harbour an ALK (anaplastic lymphoma kinase) gene rearrangement. The most common rearrangement is a paracentric inversion in the short arm of chromosome 2, resulting in the ALK-EML4 fusion gene, although other fusion partners have been identified.

Crizotinib (Xalkori®) is a small molecule tyrosine kinase inhibitor of ALK. Those cases of NSCLC which are positive for ALK rearrangement show significant benefit from treatment with crizotinib in terms of both tumour shrinkage and progression free survival compared to conventional chemotherapy. In 2011 crizotinib was FDA approved for clinical use in the USA for this molecularly defined sub-group of lung cancer.

We offer a FISH service for the detection of ALK gene rearrangement using a commercially available breakapart probe. We require an H&E slide, with the relevant area marked, to be sent along with the slides for testing in order to focus our analysis appropriately. The optimal thickness of sections for FISH testing is 3~5µm.

References

Camidge DR, Doebele RC. Treating ALK-positive lung cancer- early successes and future challenges. Nat. Rev. Clin. Oncol. 2012 May (9): 268-277



Malignant Mesothelioma

New section

Malignant mesothelioma is an aggressive tumour associated with previous exposure to asbestos and with an increasing incidence. Homozygous deletion of 9p21 (including CDKN2A), although not specific to this particular tumour type, has been reported in up to 74% of cases of mesothelioma but is not found in benign or reactive proliferations. Therefore, the detection of homozygous deletion of CDKN2A by fluorescence in situ hybridization (FISH) can be a useful diagnostic test to distinguish malignant mesothelioma from benign mesothelial proliferations.

The laboratory has validated a FISH assay for detection of homozygous deletion of CDKN2A in paraffin embedded tissue sections from suspected cases of malignant mesothelioma. Both cellular pleural effusions and tissue biopsies appear acceptable. For this test it is important that we are provided with information regarding the tumour type, an H&E slide marked with the area of interest for analysis and if possible with an informative IHC slide (e.g. calretinin), to help guide our analysis to the relevant areas.

The proportion of cells considered positive by the laboratory for reporting a homozygous deletion is $\geq 20\%$. Hemizygous deletion (loss of single copy of CDKN2A) or apparent monosomy for chromosome 9 (loss of CDKN2A and one control signal) are not sufficiently sensitive to distinguish malignant tumours and will not be reported.

References

Takeda et al, Pathology International 2010; 60: 395-399
Chung CT et al, J Clin Pathol. 2010; 63(7):630-4.



Appendix

The Christie

The Christie NHS Foundation Trust is the largest single-site cancer treatment centre of its kind in Europe, and is an international leader in cancer research and development. The hospital is based in Manchester and, as it is a specialist centre, patients are referred to The Christie from all over the North West and beyond. The Christie covers a population of 3.5 million. Over its hundred-year history, The Christie has pioneered numerous developments in cancer diagnosis and therapy. It works in close partnership with many organisations such as other NHS Trusts, Manchester Universities, Cancer Research UK and the Paterson Institute for Cancer Research.

Greater Manchester and Cheshire Haematology Malignancy Diagnostics

Greater Manchester and Cheshire Haematology Malignancy Diagnostics (GMC HMD) service formally opened in December 2007. This will be a comprehensive and coordinated service jointly provided by The Christie and Manchester Royal Infirmary (MRI) and will include an integrated molecular diagnostic service for leukaemia and lymphoma. Routine cytogenetics and FISH-based molecular cytogenetic tests will be provided by the Oncology Cytogenetics service and will be further developed as required. The GMC HMD service development will allow compliance with national strategy as expressed in the National Institute for Clinical Excellence (NICE) *Improving Outcomes Guidance for Haematological Cancers*, and has been designed to meet the needs in this regard of the Greater Manchester and Cheshire Cancer Network and has been planned with the Greater Manchester Pathology Network.

Phase 1 of HMD provides enhanced lymphoma diagnosis on paraffin-embedded tissue, including central lymphoma morphology review and fluorescence in situ hybridisation (FISH) for diagnostic translocations at The Christie and B and T-cell clonality studies by RT-PCR at MRI. The lead Histopathologist for the service collates an integrated report with overall interpretative comments. Further development of HMD is expected and will include coordination of leukaemia and myeloma diagnosis in later phases.



Review History

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